EFFECT OF HYDROGEN PEROXIDE ON SOUR CHERRY ANTHOCYANINS

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ABSTRACT

Degradation of sour cherry anthocyanins was studied at different H_2O_2 concentrations (0.233-11.63 mmol.L^{-1}) over the temperature range of 20-55C. Degradation reaction fitted to a first order kinetic model progressed very rapidly even at low H_2O_2 concentrations. Thus, the $t_{1/2}$ values at 20C varied between 111-20 h in the concentration range of 0.233-2.327 mmol.L^{-1} H_2O_2. The degradation of anthocyanins occurred at a faster rate with increasing temperature at 5.82 and 11.63 mmol.L^{-1} H_2O_2 concentrations. Between 25-55C, activation energies (E_a) were 9.53 and 10.60 kcal.mol^{-1} for 5.82 and 11.63 mmol.L^{-1} H_2O_2 concentrations, respectively. Higher E_a value at 11.63 mmol.L^{-1} H_2O_2 concentration indicated that the effect of temperature increased at higher H_2O_2 concentrations. A quadratic relationship ($y = -0.0031x^2 + 0.0218x + 0.0008$, $R^2 = 0.996$) was found between the degradation rates at 20C and H_2O_2 concentrations of 0.233-2.327 mmol.L^{-1}. According to this equation, k of 1.12 x 10^{-3} h^{-1} and $t_{1/2}$ of 26 days at 20C may be expected at 0.5 ppm (0.0147 mmol.L^{-1}) H_2O_2 concentration, i.e., the max. allowable H_2O_2 level by FDA in the finished food packages.

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INTRODUCTION

Hydrogen peroxide (H_2O_2) is the most popular chemical sterilant for treatment of plastic packaging material used in aseptic processing systems (Toledo 1975; Von Bockelmann and Von Bockelmann 1986; Mitchell 1988). FDA classifies H_2O_2 as generally recognized as safe (GRAS) and approved the use of H_2O_2 as a package sterilant in February 1981 (Code of Federal Regulations 1995a). This approval opened the doors for the use of flexible laminated cartons for consumer-size packages in place of traditional glass and can containers (Tillotson 1984; Nelson 1993).

The sterilization of the packaging material used in aseptic processing is carried out by either dipping the packaging material into a H_2O_2 bath or spraying H_2O_2 onto the polyethylene food contact surfaces. The remaining H_2O_2 is removed from the food contact surfaces by drying with sterile hot air. FDA currently limits the residual H_2O_2 in the finished food packages to 0.5 ppm (Code of Federal Regulations 1995b). However, during the sterilization of aseptic chamber or packaging material with H_2O_2, residues left on the packaging material or vapors generated during drying may get trapped inside the package upon sealing (Toledo 1986). Therefore, the residues left inside the packages may occasionally be over the FDA limit and may cause the quality losses in fruit juices. Johnson and Toledo (1975) reported a two-fold increase in the degradation rate of ascorbic acid in orange juice concentrate when the aseptic chamber was presterilized with H_2O_2 instead of steam. Also, Sonheimer and Kertesz (1952) observed the extreme susceptibility of anthocyanins to H_2O_2 in strawberry juice.

Sour cherry juice is widely consumed in Turkey and is mostly marketed as a single strength juice in carton-based laminated packages. The color loss in aseptically packaged sour cherry juice has been brought to our attention by one of the major juice producers. Therefore, this study was undertaken mainly to determine the effects of hydrogen peroxide on the degradation of sour cherry anthocyanins.

MATERIALS AND METHODS

Materials

Sour cherry (*Prunus cerasus* L.) juice concentrate was obtained from a local fruit juice company. The concentrate was kept frozen until used for analysis.

Methods

Sample Preparation and Absorption Spectra. The juice concentrate was diluted with distilled water to provide an absorbance value between 0.6-0.8 units. The diluted juice was filtered through Whatman no.1 filter paper before used for analysis.

The absorption spectra were scanned from 350 to 700 nm. The wavelength of maximum absorption was 512 nm for sour cherry anthocyanins. All absorbance readings were made against distilled water as a blank. Spectrophotometric measurements were carried out using a PYE Unicam SPG-550 UV/VIS spectrophotometer.

Effect of Temperature. The effect of temperature on sour cherry anthocyanins was studied at four temperatures (25, 35, 45 and 55C) for 5.82 and 11.63 mmol.L^{-1} H_2O_2 concentrations. The juice samples were allowed to reach the required temperature. Then, the diluted H_2O_2 solution was added rapidly to the juice samples which were made up to volume.

The absorbance of the sample solutions was measured periodically. The zero-time absorbance values were determined by preparing the samples with the same amount of distilled water instead of H_2O_2 solution. In the temperature range of 25-55C, the change in absorbance of the sample solution containing no H_2O_2 is insignificant over the time. The anthocyanin retention for each time period was calculated as percentage of zero-time absorbance readings.

Effect of H_2O_2 Concentration. The juice samples with H_2O_2 concentrations ranging from 0.233 to 2.327 mmol.L^{-1} were prepared and placed in a cold room at 20C. Samples were periodically removed from the storage and the absorbance values determined. The percentage of anthocyanin retention was calculated as described above.

RESULTS AND DISCUSSION

Degradation Kinetics

The possible mechanism for the reaction between anthocyanins and H_2O_2 was outlined by Sondheimer and Kertesz (1952). This reaction occurs in two steps: an initial reversible reaction with the formation of anthocyanin-H_2O_2 adduct, followed by a slower irreversible one. The degradation of sour cherry anthocyanins by H_2O_2 was fitted to a first order kinetic model (Fig. 1). The reaction rates (k) and half-lives ($t_{1/2}$), i.e., the time needed for 50% degradation of anthocyanins at a given H_2O_2 concentration and temperature, were calculated by the following equations:

$$\ln (A_o / A_t) = -kt \quad (1)$$

$$t_{1/2} = \text{In } 0.5/k \quad (2)$$

where A_0 is the initial absorbance of diluted fruit juice and A_t is the absorbance value after t minute incubation at a given temperature.

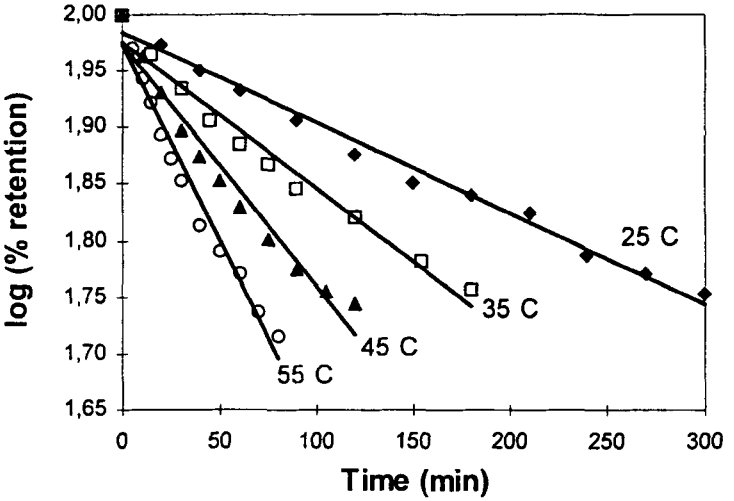

FIG. 1. DEGRADATION OF SOUR CHERRY ANTHOCYANINS IN PRESENCE OF 5.82 mmol.L^{-1} H_2O_2 CONCENTRATION AT DIFFERENT TEMPERATURES

In the temperature range of 25-55C, the k values varied between (1.84 - 8.00) x 10^{-3} min^{-1} and (2.53 - 13.00) x 10^{-3} min^{-1}, and $t_{1/2}$ values varied between 377-87 and 274-53 min for 5.82 mmol.L^{-1} and 11.63 mmol.L^{-1} H_2O_2 concentrations, respectively (Table 1).

The studies on the degradation of anthocyanins by H_2O_2 are limited. Only Sondheimer and Kertesz (1952) reported the $t_{1/2}$ values for strawberry juice anthocyanins as 6, 9 and 13 min for 77.4, 10.7 and 2.42 mmol.L^{-1} H_2O_2 concentrations at 20C. The comparison of $t_{1/2}$ values of strawberry anthocyanins to those of sour cherry anthocyanins revealed that strawberry anthocyanins were extremely susceptible to H_2O_2.

Effect of Temperature

The temperature dependence of anthocyanin degradation was determined by plotting k versus temperature (Fig. 2) and by calculating the activation energies (E_a) and temperature quotients (Q_{10}) (Table 2). As seen in Fig. 2, the effect of temperature on the degradation rates showed an increase at the higher H_2O_2 concentration. Therefore, in packaged fruit juices, greater loss of anthocyanins should be expected as residual H_2O_2 concentration and storage temperature increase.

The E_a and Q_{10} values were determined from the following equations:

$$k = k_o \cdot e^{-Ea/RT} \qquad (3)$$

$$Q_{10} = k\,(T+10) / k\,(T) \qquad (4)$$

TABLE 1.
EFFECT OF TEMPERATURE ON THE DEGRADATION OF SOUR CHERRY ANTHOCYANINS BY H_2O_2

H_2O_2 conc.(mmol.L^{-1})	Temperature (C)	k.10^3 (min^{-1})	$t_{1/2}$ (min)
5.82	25	1.84 (0.988)[a]	377
	35	2.97 (0.972)	233
	45	4.90 (0.965)	141
	55	8.00 (0.973)	87
11.63	25	2.53 (0.977)	274
	35	5.59 (0.968)	124
	45	9.40 (0.983)	74
	55	13.00 (0.954)	53

[a]Number in brackets is the determination coefficient.

The calculated E_a values were 9.53 and 10.6 kcal.mol^{-1} for 5.82 and 11.63 mmol.L^{-1} H_2O_2 concentrations, respectively. In the temperature range of 25-55C, the Q_{10} values for 5.82 mmol.L^{-1} H_2O_2 concentration did not change greatly (1.61-1.65), whereas, at 11.63 mmol.L^{-1} H_2O_2 concentration the Q_{10} values were reduced from 2.21 to 1.38 by increasing the temperature (Table 2).

In long term storage, the temperature alone is also effective on the degradation of anthocyanins. In fact, Cemeroğlu *et al.* (1994) showed that storing sour cherry concentrate at 5C rather than 20C resulted in almost 10-folds increase in $t_{1/2}$ values of sour cherry anthocyanins, i.e., from 38 to 356 days. And also, the color loss half lives of aseptically packaged cranberry juice cocktail were reported to be 112 days at 21C and 86 days at 36C (Toledo 1986). Therefore, cold storage of aseptically packaged sour cherry juice is strongly recommended to prevent anthocyanin degradation by temperature and residual H_2O_2.

Effect of Concentration

The effect of H_2O_2 concentration on anthocyanin degradation was studied at 20C (Fig. 3). As expected, the degradation of anthocyanins occurred at a faster rate when the H_2O_2 concentration increased. Between 0.233 and 2.327 mmol.L^{-1} H_2O_2 concentrations, the k and $t_{1/2}$ values varied between (6.22-34.81) x 10^{-3} h^{-1} and 111 -20 h, respectively (Table 3). A quadratic relationship was obtained between k and H_2O_2 concentrations and the equation for this relationship is expressed as:

$$y = -0.0031x^2 + 0.0218x + 0.0008 \quad (R^2 = 0.996)$$

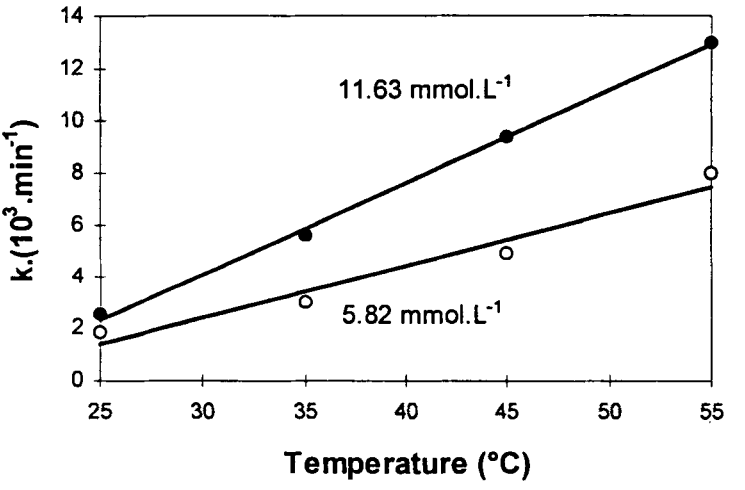

FIG. 2. EFFECT OF TEMPERATURE ON THE DEGRADATION RATE CONSTANTS OF SOUR CHERRY ANTHOCYANINS AT DIFFERENT H_2O_2 CONCENTRATIONS

TABLE 2.
E_a AND Q_{10} VALUES FOR THE DEGRADATION OF SOUR CHERRY ANTHOCYANINS BY H_2O_2

H_2O_2 conc.	E_a	Q_{10}		
(mmol.L^{-1})	(kcal.mol^{-1})	25-35C	35-45C	45-55C
5.82	9.53 (0.999)	1.61	1.65	1.63
11.63	10.60 (0.974)	2.21	1.68	1.38

[a]Number in brackets is the determination coefficients.

In contrast to our results, Sondheimer and Kertesz (1952) found a linear relationship between k and H_2O_2 concentration in strawberry anthocyanin solution in buffer. If one places 0.5 ppm (0.0147 mmol.L^{-1}) in the above equation, which is the maximum allowed level of H_2O_2 in the finished food packages by FDA, a k of 1.12 x 10^{-3} h^{-1} and $t_{1/2}$ of 26 days at 20C are calculated. Such values indicate that fruit juice anthocyanins may be degraded even at very low H_2O_2 concentrations.

In conclusion, sour cherry anthocyanins were found to be very susceptible to H_2O_2. A rapid degradation may occur even at H_2O_2 concentrations as low as FDA limit. Therefore, aseptic systems should be frequently controlled to ensure the effective removal of residual H_2O_2 from the food contact surfaces. Since the rate of

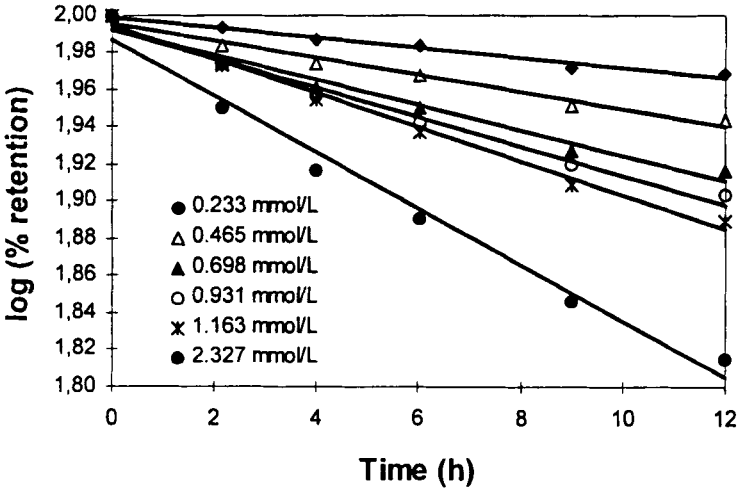

FIG. 3. EFFECT OF H_2O_2 CONCENTRATION ON THE DEGRADATION OF SOUR CHERRY ANTHOCYANINS AT 20C

TABLE 3.
EFFECT OF DIFFERENT H_2O_2 CONCENTRATIONS ON THE DEGRADATION OF SOUR CHERRY ANTHOCYANINS AT 20C

H_2O_2 conc. (mmol.L^{-1})	k.10^3 (h^{-1})	$t_{1/2}$ (h)
0.233	6.22 (0.982)[a]	111
0.465	10.59 (0.975)	65
0.698	15.56 (0.969)	45
0.931	18.09 (0.981)	38
1.163	20.96 (0.990)	33
2.327	34.81 (0.982)	20

[a]Number in brackets is the determination coefficients.

anthocyanin degradation by H_2O_2 is highly dependent on temperature, low temperature storage is recommended to maintain the bright red color of sour cherry juice.

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REFERENCES

CEMEROĞLU, B., VELIOĞLU, S. and IŞIK, S. 1994. Degradation kinetics of anthocyanins in sour cherry juice and concentrate. J. Food Sci. *59*, 1216-1218.

Code of Federal Regulations, title 21, 184.1366. 1995a. Direct food substances affirmed as generally recognized as safe. Office of the Federal Register, U.S. Government Printing Office, Washington, D.C.

Code of Federal Regulations, title 21, 178.1005. 1995b. Indirect food additives. Office of the Federal Register, U.S. Government Printing Office, Washington, D.C.

JOHNSON, R.L. and TOLEDO, R.T. 1975. Storage stability of 55° Brix orange juice concentrate aseptically packaged in plastic and glass containers. J. Food Sci. *40*, 433-434.

MITCHELL, E.L. 1988. A review of aseptic processing. Adv. Food Res. *32*, 1-37.

NELSON, P.E. 1993. Introduction. In *Principles of Aseptic Processing and Packaging*, (J.V. Chambers and P.E. Nelson, eds.) 2nd Ed., pp.1-2, The Food Processors Institute, Washington D.C.

SONDHEIMER, E. and KERTESZ, Z.I. 1952. The kinetics of the oxidation of strawberry anthocyanin by hydrogen peroxide. Food Res. *17*, 288-298.

TILLOTSON, J.E. 1984. Aseptic packaging of fruit juices. Food Technol. *38*(3), 63-66.

TOLEDO, R.T. 1975. Chemical sterilants for aseptic packaging. Food Technol. *29*(5), 102-112.

TOLEDO, R.T. 1986. Postprocesing changes in aseptically packaged beverages. J. Agric. Food Chem. *34*, 405-408.

VON BOCKELMANN, B.A.H. and VON BOCKELMANN, I.L.I. 1986. Aseptic packaging of liquid food products: A literature review. J. Agric. Food Chem. *34*, 384-392.